NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 434

TOXICOLOGY AND CARCINOGENESIS STUDIES OF 1,3-BUTADIENE

(CAS NO. 106-99-0)

IN B6C3F$_1$ MICE

(INHALATION STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge while supplies last from the NTP Central Data Management Office, NIEHS, P.O. Box 12233, MD-A0-01, Research Triangle Park, NC 27709 (919-541-1371).

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF 1,3-BUTADIENE

(CAS NO. 106-99-0)

IN B6C3F$_1$ MICE

(INHALATION STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

May 1993

NTP TR 434

NIH Publication No. 93-3165

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

CONTRIBUTORS

National Toxicology Program
Evaluated and interpreted results and reported findings

C.J. Alden, Ph.D.
G.A. Boorman, D.V.M., Ph.D.
D.A. Bridge, B.S.
S.L. Eustis, D.V.M., Ph.D.
T.J. Goehl, Ph.D.
R.A. Griesemer, D.V.M., Ph.D.
J.R. Hailey, D.V.M.
J.K. Haseman, Ph.D.
R.L. Melnick, Ph.D.
G.N. Rao, D.V.M., Ph.D.
C.C. Shackelford, D.V.M., M.S., Ph.D.
D.B. Walters, Ph.D.
K.L. Witt, M.S., Oak Ridge Associated Universities

Battelle Pacific Northwest Laboratories
Conducted studies, evaluated pathology findings

B.J. Chou, D.V.M., Ph.D., Principal Investigator
R.A. Miller, D.V.M., Ph.D.

Experimental Pathology Laboratories, Inc.
Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
H.R. Brown, D.V.M., M.S.
B.F. Hamilton, D.V.M., Ph.D.

Integrated Laboratory Systems
Prepared quality assurance audits

S.L. Smith, J.D., Principal Investigator

NTP Pathology Working Group
Evaluated slides, prepared pathology report for mice (26 July 1991)

J.R. Leininger, D.V.M., Ph.D., Chair
Pathology Associates, Inc.
M.R. Elwell, D.V.M., Ph.D.
National Toxicology Program
S.L. Eustis, D.V.M., Ph.D.
National Toxicology Program
J.R. Hailey, D.V.M.
National Toxicology Program
R.A. Miller, D.V.M., Ph.D.
Battelle Pacific Northwest
S.R. Qureshi, B.V.Sc., Ph.D.
Sandoz Ltd.
R.C. Sills, D.V.M., Ph.D.
National Toxicology Program
K. Yoshitomi, D.V.M., Ph.D.
Experimental Pathology Laboratories

Biotechnical Services, Inc.
Prepared Technical Report

D.D. Lambright, Ph.D., Principal Investigator
G.F. Corley, D.V.M.
T.A. King-Hunter, B.S.
W.D. Sharp, B.A., B.S.

CONTENTS

ABSTRACT

$$CH_2=CH-CH=CH_2$$

1,3-BUTADIENE

CAS No. 106-99-0

Chemical Formula: C_4H_6 Molecular Weight: 54.09

Synonyms: α,γ-Butadiene; bivinyl; divinyl; erythrene; vinylethylene; biethylene; pyrrolylene

1,3-Butadiene is produced in large volumes for use in the manufacture of synthetic rubber and of thermoplastic resins. In previous inhalation studies conducted by the NTP (NTP, 1984) there was clear evidence of multiple organ carcinogenicity in male and female mice exposed to 625 or 1,250 ppm 1,3-butadiene for 60 or 61 weeks. To better characterize exposure-response relationships for neoplasms and nonneoplastic lesions, toxicology and carcinogenesis studies were conducted by exposing groups of male and female $B6C3F_1$ mice to air containing 1,3-butadiene (greater than 99% pure) for up to 2 years. An additional study in male $B6C3F_1$ mice, in which exposure to 1,3-butadiene was stopped after limited exposure periods (13, 26, 40, or 52 weeks), was performed to assess the effects of varying concentration and duration of exposure on the incidences of 1,3-butadiene-induced neoplasms. *In vitro* genetic toxicology studies were conducted in *Salmonella typhimurium* and mouse lymphoma cells. *In vivo* genetic effects were assayed in germ cells of male *Drosophila melanogaster* and in bone marrow and peripheral blood cells of $B6C3F_1$ mice.

2-Year Studies: Groups of 70 male and 70 female mice were exposed to air containing 0, 6.25, 20, 62.5, or 200 ppm 1,3-butadiene for 6 hours per day, 5 days per week for up to 2 years; groups of 90 male and 90 female mice were exposed to 625 ppm 1,3-butadiene on the same schedule. Up to 10 animals from each group were examined after 9 and 15 months of exposure.

Survival and Body Weight in the 2-Year Studies: Two-year survival was decreased for males and females exposed to concentrations of 20 ppm or above, primarily due to the development of chemical-related malignant neoplasms. No female mice exposed to 200 or 625 ppm or males exposed to 625 ppm survived to the end of the studies (males: 35/50, 39/50, 24/50, 22/50, 4/50, 0/70; females: 37/50, 33/50, 24/50, 11/50, 0/50, 0/70). Mean body weights of exposed male and female mice were similar to those of the controls.

Hematologic Effects in the 2-Year Studies: Hematologic parameters were evaluated after 9 and 15 months of exposure. At 9 months, decreases in erythrocyte counts, hemoglobin concentration, and packed red cell volume were observed in male mice exposed to 62.5 ppm or above and in female mice exposed to 200 or 625 ppm. Mean erythrocyte volume was increased in male mice exposed to 625 ppm and in females exposed to 200 or 625 ppm. At 15 months, decreases in erythrocyte counts, hemoglobin concentration, and packed red cell volume and increases in mean erythrocyte volume were observed in male and female mice exposed to 625 ppm.

Neoplasms and Nonneoplastic Lesions in the 2-Year Studies: Exposure of mice to 1,3-butadiene induced benign and malignant neoplasms at multiple sites. Statistically significant increases in the incidences of neoplasms at one or more sites were seen at concentrations of 20 ppm and higher in males and 6.25 ppm and higher in females. There was no exposure level in this study at which a significant carcinogenic response was not observed. Statistically significant increases occurred in the incidences of malignant lymphoma; histiocytic sarcoma; cardiac hemangiosarcoma; harderian gland adenoma; hepatocellular adenoma and carcinoma; alveolar/bronchiolar adenoma and carcinoma; mammary gland carcinoma, adenoacanthoma, and malignant mixed tumor (females only); benign and malignant ovarian granulosa cell tumor; and forestomach squamous cell papilloma and carcinoma.

Low incidences of uncommon neoplasms also occurred in exposed male and female mice, including intestinal carcinomas in males, renal tubule adenomas in males and females, skin sarcomas (all types combined) in females, and Zymbal's gland adenomas and carcinomas in females.

Lymphocytic lymphomas appeared as early as week 23 and were the principal cause of death of male and female mice exposed to 625 ppm 1,3-butadiene. The early and extensive development of lethal lymphocytic lymphomas in mice exposed to 625 ppm resulted in a reduced number of mice at risk for neoplasms developing later at other sites. Exposure-response relationships for 1,3-butadiene-induced neoplasms were more clearly characterized at concentrations below 625 ppm and after adjustment for intercurrent mortality.

Increased incidences of nonneoplastic lesions in exposed mice included bone marrow atrophy; testicular atrophy; ovarian atrophy, angiectasis, germinal epithelial hyperplasia, and granulosa cell hyperplasia; uterine atrophy; cardiac endothelial hyperplasia and mineralization; alveolar epithelial hyperplasia; forestomach epithelial hyperplasia; and harderian gland hyperplasia.

Stop-Exposure Study: The stop-exposure study consisted of groups of 50 male mice exposed to 1,3-butadiene at concentrations of 200 ppm for 40 weeks, 625 ppm for 13 weeks, 312 ppm for 52 weeks, or 625 ppm for 26 weeks. After the exposures were completed, these groups were placed in control chambers for the remainder of the 2-year study. The total exposure of 1,3-butadiene (concentration times duration of exposure) of the 13- and 40-week stop-exposure groups was approximately 8,000 ppm · weeks, while that of the 26- and 52-week stop-exposure groups was approximately 16,000 ppm · weeks.

The survival of all stop-exposure groups was markedly lower than that of the controls. The incidences of lymphocytic lymphoma, histiocytic sarcoma, cardiac hemangiosarcoma, alveolar/bronchiolar adenoma and carcinoma, forestomach squamous cell papilloma and carcinoma, hepatocellular adenoma, harderian gland adenoma and adenocarcinoma, and preputial gland carcinoma were significantly increased. Neoplasms were induced at most of these sites after only 13 weeks of exposure to 1,3-butadiene. Additionally, low numbers of malignant gliomas and neuroblastomas of the brain and Zymbal's gland carcinomas occurred in one or more stop-exposure groups.

At similar total exposures, the incidence of lymphocytic lymphoma was greater with exposure to a higher concentration of 1,3-butadiene for a short time compared with exposure to a lower concentration for an extended period (34% at 625 ppm for 13 weeks versus 12% at 200 ppm for 40 weeks; 60% at 625 ppm for 26 weeks versus 8% at 312 ppm for 52 weeks).

Genetic Toxicology: 1,3-Butadiene has been tested both *in vitro* and *in vivo* for mutagenic activity. *In vitro*, positive results were obtained in the *Salmonella typhimurium* gene mutation assay with strain TA1535; mutagenic activity was not observed in other *S. typhimurium* strains (TA100, TA97, and TA98). 1,3-Butadiene was negative in the mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y cells with and without S9.

In vivo, 1,3-butadiene did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster*; however, it did induce significant increases in chromosomal aberrations and sister chromatid exchanges in bone marrow cells of mice exposed for 2 weeks by inhalation. In addition, significant increases in micronucleated erythrocytes were observed in peripheral blood samples obtained

from male and female mice exposed to 1,3-butadiene for 2 or 13 weeks or 15 months by inhalation.

Conclusions: The previous inhalation studies of 1,3-butadiene in male and female $B6C3F_1$ mice provided *clear evidence of carcinogenicity** at exposure concentrations of 625 or 1,250 ppm. The present inhalation studies — 2-year exposures of 6.25, 20, 62.5, 200, or 625 ppm or shorter duration exposures of 200, 312, or 625 ppm — provide a better characterization of the concentration-dependent responses for 1,3-butadiene-induced neoplasms and nonneoplastic lesions. The present studies confirmed the *clear evidence of carcinogenicity* of 1,3-butadiene in male $B6C3F_1$ mice based on increased incidences of neoplasms in the hematopoietic system, heart, lung, forestomach, liver, harderian gland, preputial gland, brain, and kidney. There was *clear evidence of carcinogenicity* of 1,3-butadiene in female $B6C3F_1$ mice based on increased incidences of neoplasms in the hematopoietic system, heart, lung, forestomach, liver, harderian gland, ovary, and mammary gland.

Low incidences of intestinal carcinomas in male mice, Zymbal's gland carcinomas in male and female mice, and renal tubule adenomas and skin sarcomas in female mice may also have been related to administration of 1,3-butadiene.

* Explanation of Level of Evidence of Carcinogenic Activity is on page 11. A summary of peer review comments and the public discussion on this Technical Report appears on page 13.

Summary of the 2-Year Carcinogenicity and Genetic Toxicology Studies of 1,3-Butadiene

	Male B6C3F$_1$ Mice		Female B6C3F$_1$ Mice
	(2-Year Study)	(Stop-Exposure Study)	
Doses	0, 6.25, 20, 62.5, 200, or 625 ppm by inhalation for 6 hours daily, 5 days per week, for 103 weeks	200 ppm for 40 weeks, 312 ppm for 52 weeks, 625 ppm for 13 weeks, or 625 ppm for 26 weeks by inhalation for 6 hours daily, 5 days per week	0, 6.25, 20, 62.5, 200, or 625 ppm by inhalation for 6 hours daily, 5 days per week, for 103 weeks
Body weights	Exposed groups similar to controls	Exposed groups similar to controls	Exposed groups similar to controls
2-Year survival rates	35/50, 39/50, 24/50, 22/50, 4/50, 0/70	9/50, 1/50, 5/50, 0/50	37/50, 33/50, 24/50, 11/50, 0/50, 0/70
Nonneoplastic effects	Bone marrow: atrophy (0/50, 0/50, 0/50, 0/48, 0/49, 23/73) Heart: endothelial hyperplasia (0/50, 1/49, 0/50, 2/48, 4/48, 5/73); mineralization (0/50, 0/49, 0/50, 1/48, 3/48, 20/73) Alveolar epithelium: hyperplasia (2/50, 9/50, 6/50, 13/49, 17/50, 12/73) Forestomach epithelium: hyperplasia (4/50, 3/50, 3/50, 6/48, 4/48, 40/72) Harderian gland: hyperplasia (1/50, 3/49, 4/50, 6/47, 8/47, 5/40) Testicle: atrophy (1/50, 3/50, 4/50, 2/48, 6/49, 53/72)	Heart: endothelial hyperplasia (6/50, 3/50, 7/50, 7/50); mineralization (0/50, 6/50, 9/50, 14/50) Alveolar epithelium: hyperplasia (18/50, 14/50, 10/50, 11/50) Forestomach epithelium: hyperplasia (10/48, 20/48, 8/50, 15/50) Harderian gland: hyperplasia (4/48, 6/48, 3/42, 7/36) Testicle: atrophy (5/50, 3/50, 3/50, 5/50)	Bone marrow: atrophy (0/50, 0/49, 0/48, 0/49, 0/50, 11/79) Heart: endothelial hyperplasia (0/50, 2/50, 1/50, 4/49, 5/50, 8/80); mineralization (0/50, 2/50, 0/50, 2/49, 2/50, 11/80) Alveolar epithelium: hyperplasia (5/50, 5/50, 3/50, 9/50, 11/50, 11/78) Forestomach epithelium: hyperplasia (4/50, 5/49, 4/47, 7/48, 14/50, 47/79) Liver: hepatocellular foci (8/49, 14/49, 19/50, 12/50, 5/50, 4/80) Harderian gland: hyperplasia (1/50, 5/49, 9/48, 4/49, 4/49, 7/66) Ovary: angiectasis (4/49, 6/49, 3/48, 13/50, 14/50, 17/79); granulosa cell hyperplasia (1/49, 0/49, 2/48, 3/50, 4/50, 2/79); germinal epithelial hyperplasia (2/49, 3/49, 8/48, 15/50, 14/50, 18/79); atrophy (4/49, 19/49, 32/48, 42/50, 43/50, 69/79) Uterus: atrophy (1/50, 0/49, 1/50, 1/49, 8/50, 41/78)

(continued)

Summary of the 2-Year Carcinogenicity and Genetic Toxicology Studies of 1,3-Butadiene (continued)

	Male $B6C3F_1$ Mice (2-Year Study)	Male $B6C3F_1$ Mice (Stop-Exposure Study)	Female $B6C3F_1$ Mice
Neoplastic effects	Lymphoma (all lymphomas) (4/50, 2/50, 4/50, 6/50, 2/50, 51/73) Lymphocytic lymphoma (2/50, 0/50, 2/50, 4/50, 2/50, 49/73) Histiocytic sarcoma (0/50, 0/50, 4/50, 5/50, 7/50, 4/73) Heart: hemangiosarcoma (0/50, 0/49, 1/50, 5/48, 20/48, 4/73) Lung: alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma (21/50, 23/50, 19/50, 31/49, 35/50, 3/73) Forestomach: squamous cell papilloma or squamous cell carcinoma (1/50, 0/50, 0/50, 1/50, 8/50, 4/73) Liver: hepatocellular adenoma or carcinoma (21/50, 23/50, 30/50 25/48, 33/48, 5/72) Harderian gland: adenoma or carcinoma (6/50, 7/50, 9/50, 20/50, 31/50, 6/73) Preputial gland: carcinoma (0/50, 0/50, 0/50, 0/50, 5/50, 0/73) Kidney: renal tubule adenoma (0/50, 1/50, 0/50, 3/48, 1/49, 0/73)	Lymphoma (all lymphomas) (8/50, 8/50, 22/50, 33/50) Lymphocytic lymphoma (6/50, 4/50, 17/50, 30/50) Histiocytic sarcoma (5/50, 7/50, 2/50, 2/50) Heart: hemangiosarcoma (15/50, 33/50, 7/50, 13/50) Lung: alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma (36/50, 32/50, 28/50, 17/50) Forestomach: squamous cell papilloma or squamous cell carcinoma (3/50, 9/50, 7/50, 10/50) Liver: hepatocellular adenoma (27/49, 19/50, 19/49, 11/50) Harderian gland: adenoma or carcinoma (27/50, 30/50, 23/50, 13/50) Preputial gland: carcinoma (1/50, 4/50, 4/50, 3/50) Kidney: renal tubule adenoma (4/48, 3/49, 1/50, 1/50) Brain: malignant glioma (0/50, 0/50, 2/50, 1/50); neuroblastoma (0/50, 0/50, 2/50, 0/50)	Lymphoma (all lymphomas) (6/50, 12/50, 11/50, 7/50, 9/50, 32/80) Lymphocytic lymphoma (1/50, 3/50, 6/50, 3/50, 8/50, 31/80) Histiocytic sarcoma (3/50, 2/50, 7/50, 4/50, 7/50, 4/80) Heart: hemangiosarcoma (0/50, 0/50, 0/50, 1/49, 21/50, 23/80) Lung: alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma (4/50, 15/50, 19/50, 24/50, 25/50, 22/78) Forestomach: squamous cell papilloma or squamous cell carcinoma (0/50, 0/50, 3/50, 2/50, 4/50, 22/80) Liver: hepatocellular adenoma or carcinoma (15/49, 14/49, 15/50, 19/50, 16/50, 2/80) Harderian gland: adenoma or carcinoma (8/50, 10/50, 7/50, 15/50, 20/50, 9/80) Ovary: benign or malignant granulosa cell tumor (1/49, 0/49, 1/48, 9/50, 8/50, 6/79); adenoma or benign mixed tumor (2/49, 4/49, 1/48, 4/50, 6/50, 2/79) Mammary gland: adeno-acanthoma, carcinoma, or malignant mixed tumor (0/50, 2/50, 4/50, 12/50, 15/50, 16/80)

(continued)

Summary of the 2-Year Carcinogenicity and Genetic Toxicology Studies of 1,3-Butadiene (continued)

	Male $B6C3F_1$ Mice (2-Year Study)	Male $B6C3F_1$ Mice (Stop-Exposure Study)	Female $B6C3F_1$ Mice
Uncertain findings	Small intestine: carcinoma (0/50, 1/50, 1/50, 1/50, 2/50, 0/73)	Zymbal's gland: carcinoma (1/50, 0/50, 2/50, 2/50)	Kidney: renal tubule adenoma (0/49, 0/49, 0/48, 0/50, 2/50, 0/80) Skin, subcutaneous tissue: neurofibrosarcoma or sarcoma (1/50, 2/50, 3/50, 5/50, 3/50, 3/80) Zymbal's gland: adenoma or carcinoma (0/50, 0/50, 0/50, 0/50, 0/50, 2/80)
Level of evidence of carcinogenic activity	Clear evidence		Clear evidence
Genetic toxicology			
Salmonella typhimurium gene mutation:	Positive in strain TA1535 Negative in strains TA100, TA97, and TA98		
Mouse lymphoma gene mutation:	Negative with and without S9		
Sex-linked recessive lethal mutations *Drosophila melanogaster*:	Negative by inhalation		
Chromosomal aberrations Mouse bone marrow *in vivo*:	Positive		
Sister chromatid exchanges Mouse bone marrow *in vivo*:	Positive		
Micronuclei Mouse peripheral blood erythrocytes *in vivo*:	Positive		

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on 1,3-butadiene on November 21, 1991, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

Curtis D. Klaassen, Ph.D., Chair
Department of Pharmacology and Toxicology
University of Kansas Medical Center
Kansas City, KS

Paul T. Bailey, Ph.D.
Toxicology Division
Mobil Oil Corporation
Princeton, NJ

Louis S. Beliczky, M.S., M.P.H.
Department of Industrial Hygiene
United Rubber Workers International Union
Akron, OH

Gary P. Carlson, Ph.D.
Department of Pharmacology and Toxicology
Purdue University
West Lafayette, IN

Kowetha A. Davidson, Ph.D.
Health and Safety Research Division
Oak Ridge National Laboratory
Oak Ridge, TN

Harold Davis, D.V.M., Ph.D.*
School of Aerospace Medicine
Brooks Air Force Base, TX

Robert H. Garman, D.V.M.
Consultants in Veterinary Pathology
Murrysville, PA

Jay I. Goodman, Ph.D., Principal Reviewer
Department of Pharmacology and Toxicology
Michigan State University
East Lansing, MI

David W. Hayden, D.V.M., Ph.D.
Department of Veterinary Pathobiology
College of Veterinary Medicine
University of Minnesota
St. Paul, MN

Daniel S. Longnecker, M.D.*
Department of Pathology
Dartmouth Medical School
Lebanon, NH

Barbara McKnight, Ph.D.
Department of Biostatistics
University of Washington
Seattle, WA

Ellen K. Silbergeld, Ph.D.*
University of Maryland Medical School
Baltimore, MD

Matthew J. van Zwieten, D.V.M., Ph.D., Principal Reviewer
Department of Safety Assessment
Merck, Sharp & Dohme Research Laboratories
West Point, PA

Lauren Zeise, Ph.D., Principal Reviewer
California Department of Health Services/RCHAS
Berkeley, CA

*Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On November 21, 1991, the draft Technical Report on the toxicology and carcinogenesis studies of 1,3-butadiene received public review by the National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. R.L. Melnick, NIEHS, introduced the toxicology and carcinogenesis studies of 1,3-butadiene in B6C3F$_1$ mice by discussing the uses of the chemical and rationale for study, including the previous NTP study in mice; describing the experimental design for both standard and stop-exposure studies, which were intended to assess the relationship of exposure duration versus concentration on carcinogenicity; reporting on survival and toxicity, especially to the hematopoietic system and gonads in both sexes; and presenting data on neoplasms and nonneoplastic lesions caused by 1,3-butadiene at multiple sites in both sexes. Dr. Melnick reported on the expression of K-*ras* oncogenes in liver neoplasms and said that the K-*ras* is the most commonly detected oncogene in human cancers. Dr. C.C. Shackleford, NIEHS, provided a morphologic description of hemangiosarcomas of the heart induced by 1,3-butadiene. The proposed conclusions were that the present studies provided a better characterization of the concentration-dependent responses for 1,3-butadiene-induced neoplasms and nonneoplastic lesions, and confirmed the *clear evidence of carcinogenicity* of 1,3-butadiene in male and female B6C3F$_1$ mice.

Dr. Goodman, a principal reviewer, agreed with the proposed overall conclusions in male and female mice but disagreed with the inclusion of brain and kidney neoplasms in males and liver neoplasms in females as support for the level of evidence. He said the last sentence should read "equivocal evidence of carcinogenicity" instead of "low incidence of" and the reference to Zymbal's gland carcinomas in males should be omitted. Dr. Melnick thought that for low numbers of rare neoplasms "low incidence" was meaningful; however, another wording would be considered. Dr. Goodman thought that the conclusions for the stop-exposure study should be presented separately from those for the 2-year studies. Dr. Melnick noted that the results are presented and analyzed separately but, in evaluating the effect of 1,3-butadiene on an organ, the thinking was that all of the evidence should be brought to bear in drawing conclusions. Dr. Goodman asked that justification be given for the use of sex-linked recessive lethal mutations in *Drosophila melanogaster* and the micronucleus test. Dr. E. Zeiger, NIEHS, explained that the *D. melanogaster* assay is extremely predictive for carcinogenicity as there are very few false positives and that the micronucleus test is the only simple measure of somatic mutations *in vivo*.

Dr. Zeise, the second principal reviewer, agreed with the proposed conclusions. Because the study was designed to look at the issue of dose response, she thought a more extensive analysis of the dose-response data should be included, especially pertaining to the shape of the curve at lower doses. Dr. Melnick said some discussion could be given about the shape of the dose-response curve and the Poly-3 test used to provide neoplasm rates adjusted for intercurrent mortality. Dr. J.K. Haseman, NIEHS, expressed concern that mathematical modeling of the data might lead to extrapolation and risk assessment calculations, activities that are normally the purview of the regulatory agencies. Dr. Zeise noted that others are already using the NTP data for these purposes. She suggested that NTP not extrapolate, but evaluate the shape of the dose-response curve within the range of observations, because the study was designed to explore the dose response and NTP has the expertise to perform such statistical evaluations.

Dr. van Zwieten, the third principal reviewer, agreed with the proposed conclusions. He thought there should be a statement in the conclusions to the effect that a carcinogenic response was induced at all exposure levels. Also, a comment about duration of exposure necessary for a carcinogenic response in the stop-exposure study would be appropriate. Dr. Melnick said that statements would be brought forward to the Abstract.

Mr. Beliczky reported that the data from these studies had been recently used by NIOSH in conducting a risk assessment and the results have been provided to the Department of Labor for potential regulatory action by OSHA on allowable exposure

levels. Dr. Garman asked if separate classifications of lymphomas reflect the current recommendation of the NTP. Dr. S.L. Eustis, NIEHS, said accurate distinctions between types were difficult to make and of little value. Rather, identifying whether the lymphomas originated in the thymus or elsewhere was most useful.

Dr. Goodman moved that the Technical Report on 1,3-butadiene be accepted but with the conclusions for the 2-year studies separated from those for the stop-exposure study by inserting "chronic exposure to" in front of "1,3-butadiene" in the statements for male and female mice. "Brain" and "kidney" would be deleted from the listing for male mice and "liver" from the listing for female mice. Then a conclusion for the stop-exposure study would be added: "There was *clear evidence of carcinogenicity* of 1,3-butadiene in the start/stop study in $B6C3F_1$ mice based on increased incidences of neoplasms in the hematopoietic system, lung, forestomach, and harderian gland." Finally in the last sentence, "low incidences" would be replaced with "marginal increases." The motion was tabled for lack of a second. Dr. Zeise moved that the Technical Report on 1,3-butadiene be accepted with the revisions discussed and with the conclusions as written for male and female $B6C3F_1$ mice, *clear evidence of carcinogenicity*. Mr. Beliczky seconded the motion, and it was accepted by eight yes votes to one no vote (Dr. Goodman) with one abstention (Dr. Bailey).